Inheritance of Beta Carotene Content in the Wild Tomato Species *Lycopersicon cheesmanii*

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Beta carotene and lycopene, the two principal colored carotenoids present in tomato fruit, were measured in mature fruit of parental, F_1, F_2, and backcross populations derived from an initial cross of an orange-fruited accession of the wild tomato species *Lycopersicon cheesmanii* Riley with the cultivated tomato *L. esculentum* Mill. Segregation patterns for fruit pigmentation and percentage beta carotene in F_1, F_2, and backcross populations fit expected models for a single dominant gene conditioning a high percentage of beta carotene and resultant orange fruit pigmentation. The inheritance of beta carotene in *L. cheesmanii* paralleled that of the *B* gene described in *L. hirsutum*. Variation in colored carotenoid content indicated a parental influence on total lycopene and beta carotene content and suggested independent genetic control of these carotenoid levels.

Wild relatives of the cultivated tomato, *Lycopersicon esculentum* Mill., are a valuable source of genes for tomato improvement (Rick 1982). Nine species are recognized within the genus *Lycopersicon* (Rick 1979). One of three colored-fruited species, *L. cheesmanii* Riley, is limited in its natural habitat to the islands of the Galapagos archipelago. All accessions of *L. cheesmanii* are self-compatible and reciprocally hybridize with tomato. *L. cheesmanii* f. *minor* bears subspecies status (Muller 1940) and is differentiated from the type species by its ornate and highly subdivided leaflets. This wild species is regarded as a valuable source of salt tolerance (Rush and Epstein 1981) and high soluble solids (Garvey and Hewitt 1984; Poysa 1992). Rick (1956) noted *L. cheesmanii* accessions with orange fruit pigmentation attributable to the presence of high levels of beta carotene.

Red fruit color typical of the cultivated tomato is due to the accumulation of lycopene as the principal fruit carotenoid. Total fruit carotenoid content typically varies from 70 μg to 190 μg per gram fresh weight of fruit tissue. In red pigmented tomato fruit, lycopene constitutes 90–95% of the total colored carotenoid content, with beta carotene comprising the balance. The carotenes phytoene and phytofluene, which are colorless acyclic precursors of lycopene and beta carotene, typically constitute ~5%–25% of the total carotenoids. Additional minor pigments present in red-fruited tomatoes include neurosporene, and gamma- and zeta-carotene (Gross 1991; Khachik et al. 1992).

Lincoln and Porter (1950) described a single dominant gene, *B*, from the green-fruited species *L. hirsutum* that conditions the accumulation of high levels of beta carotene when introduced into *L. esculentum*. Beta carotene is accumulated at the expense of lycopene, resulting in orange-pigmented fruit. The beta modifier gene, Mo_B, modulates the relative proportion of beta carotene to lycopene in tomato (Tomes et al. 1954). More than 90% of the total carotenoids are typically accumulated as beta carotene when *B* is present with Mo_B. In combination with the dominant form of the modifier Mo_B^+, lycopene and beta carotene are present in approximately equal proportions. High beta carotene cultivars with a 10-fold increase in beta carotene content relative to red-fruited types were developed by Tomes (1958) and Tigchelaar and Tomes (1974) via introgression of *B* from *L. hirsutum* into *L. esculentum*. Use of these cultivars has been limited to the home garden. At present, orange-pigmented cultivars developed for commercial production derive their distinctive pigmentation from the action of the recessive tangerine (*t*) allele. Although orange pigmented, cultivars containing *t* accumulate increased quantities of zeta-carotene, proneurosporene, and prolycopene, which have no provitamin A potential.

There is ample justification for genetic improvement of provitamin A content in tomato and other horticultural crops. In addition to the role of carotenoids in plants as accessory pigments and protectants of the photosynthetic apparatus, carotenoids also serve as vitamin A precursors in man and other animal species. Vitamin A deficiency has been described as one of the most widespread and serious nutritional disorders to afflict humans (World Health Organization 1982). Recent epidemiological evidence suggests a positive association of increased dietary consumption of vegetables rich in beta carotene with reduced incidence of certain cancers (Doll 1990). Although low in provitamin A carotenoids relative to beta carotene–rich vegetables such as carrot, the common red, cultivated tomato makes a significant contribution (5%) (Simon 1992) to the dietary intake of vitamin A in the U.S. diet by simple virtue of the volume of fresh and processed tomato products that are consumed.

This article details the inheritance of a gene from the wild tomato species *L. cheesmanii* that conditions the accumulation of high levels of beta carotene in tomato fruit. Genetic control of total colored carotenoid content (beta carotene + lycopene) and the inheritance of beta carotene in *L. cheesmanii* relative to the *B* gene from the wild, green-fruited tomato species is discussed.

Table 1. **Beta carotene and lycopene content in parental, F_1, BC_1, and F_2 populations derived from the cross of *L. esculentum* cv. Floradade × *L. cheesmanii*, LA317**

Generation[a]	Color	Beta carotene (μg/g fr. wt.)[b]	Lycopene (μg/g fr wt)	Lycopene + beta carotene (μg/g fr. wt.)	Beta carotene (%)
P_1	Red	2 21 B	31 06 A	33.27 A	7.29 D
P_2	Orange	20.69 A	0.47 B	21 16 B	97.71 A
F_1	Orange	18 61 A	2.20 B	20.81 B	91.26 AB
BC_1	Overall	12.87 A	23.49 A	36 36 A	40.92 C
	Orange	23.99 a	6.54 a	30.53 a	79.90 a
	Red	4.23 b	36.67 b	40 90 b	10.60 b
F_2	Overall	20 91 A	8 04 B	28.95 AB	71.34 B
	Orange	24.79 a	4.34 a	29.12 a	84.40 a
	Red	5 72 b	22 57 b	28.28 a	20.15 b

[a] See Figure 1 for population descriptions.

[b] Uppercase letters denote separation of means within each column (Duncans multiple range test at $P \leq$ 05). Lower case letters denote separation of means for each generation within a column for orange and red fruit classes (Cochrans approximate *t* test, $P \leq .05$, Cochran 1964).

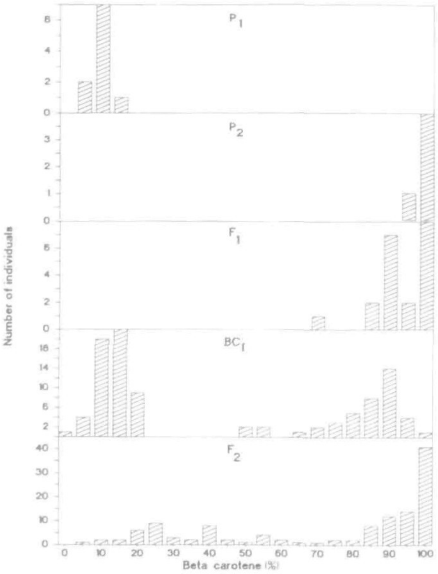

Figure 1. Beta carotene content (percentage of total colored carotenoid content; i e., lycopene + beta carotene) population distributions for P_1, P_2, F_1, BC_1, and F_2 generations (P_1 = *L. esculentum* cv. Floradade, P_2 = *L. cheesmanii*, LA317; P_1 was utilized as the recurrent parent for the BC_1 generation).

Materials and Methods

Plant Materials

L. esculentum cv. Floradade and *L. cheesmanii* f. *minor* LA 317 (Tomato Genetics Stock Center) were used as female (P_1) and male (P_2) parents, respectively. *L. esculentum* cv. Floradade served as the recurrent parent in the backcross populations. Seed of F_1, F_2, and BC_1 populations were produced on greenhouse grown plants. These plants were grown in 5-liter pots in a 6:3:2 (v:v:v) steam-treated soil, peat, and perlite mixture with regular applications of water-soluble fertilizer. Controlled cross-pollinations were performed via transfer of pollen to emasculated flowers of the cultivated parent for production of F_1 and BC_1 generation seed. F_1 plants were allowed to self-pollinate for F_2 seed production. Fruit used for carotenoid analysis were harvested from field grown plants produced on sandy loam soils at Beltsville, Maryland. Field plants were grown from 5-week-old seedlings transplanted at 61-cm spacings to raised beds 1.5 m center to center and 15 cm high. Standard horticultural practices for tomato production and pest control in Maryland were followed (University of Maryland 1991). Supplemental overhead irrigation was supplied as needed. Before harvest, ripe fruit color was scored on individual plants using Royal Horticultural Society color charts (Royal Horticultural Society 1966) for reference.

Sample Preparation and Carotene Extraction

A representative section (halved small fruit; quartered large fruit) of fully ripened fruit collected from individual plants was sampled the day of collection and frozen at −25°C. Samples were stored at −25°C up to 2-3 weeks before carotene extraction. Immediately before extraction, frozen samples were lyophilized overnight in darkness and then held at −25°C in airtight bags. Lyophilized samples were trimmed to <1 g dry wt and homogenized at 25,000 rpm for 3 min in chilled (2°C–5°C) hexane using a Virtis Cyclone homogenizer. Extracts were sequentially filtered through Whatman no. 2 filter paper discs and dried by passage through 35 g–40 g of anhydrous granular Na_2SO_4. Samples were diluted to 100 ml of final volume with hexane, and 4-ml aliquots filtered through 0.45 μm membrane filters. Filtered extracts were stored at −25°C in tightly capped amber vials up to 4 days before HPLC analysis.

Carotene Chromatography

Carotenes were separated via HPLC on a Whatman Partisil 5 ODS-3 column utilizing a mobile phase of acetonitrile/methanol/methylene chloride/hexane (85:10:2.5:2.5) (Khachik et al. 1989) at a flow rate of 1 ml min^{-1}. Apo-8′-carotenal was used as an internal standard. Peak absorption was monitored at 450 nm with a Waters 484 absorbance detector, and peak areas measured using a Waters data module. Peak areas were quantified using beta carotene and lycopene standards of known concentration.

Results and Discussion

Carotenoid content was evaluated in fruit from individual plants of parental, F_1, F_2, and BC_1 populations derived from an initial cross of *L. esculentum* (P_1) × *L. cheesmanii* (P_2). Analysis of lycopene and beta carotene, the two principal colored carotenoids present in tomato fruit, demonstrated the accumulation of high levels of beta carotene in the orange pigmented wild parent *L. cheesmanii* in contrast with the cultivated *L. esculentum* parent which stored predominantly lycopene (Table 1 and Figure 1). Whereas beta carotene comprised ~97% of these colored carotenoids in *L. cheesmanii*, lycopene accounted for nearly 94% of colored carotenoids in *L. esculentum*.

F_1 progeny derived from the original cross of *L. esculentum* and *L. cheesmanii* were orange pigmented and accumulated high levels of beta carotene similar to those noted in the wild parent (Table 1 and Figure 1). Progeny obtained from the backcross to *L. esculentum* displayed a wide range of lycopene and beta carotene content but subdivided ~1:1 into two discreet phenotypic classes based on percentage beta carotene and hence red or orange fruit pigmentation. Analysis of F_2 progeny demonstrated a continuous distribution for lycopene and beta carotene skewed toward a greater number of individuals with increased beta carotene levels. Visual ratings of fruit color in the field and subsequent laboratory analysis of fruit lycopene and beta carotene content indicated that ~35% beta carotene was required to elicit a well-defined orange phenotype. The F_2 fruit pigmentation profiles approximate a 3:1 distribution consistent with the presence of a single dominant gene conditioning a high percentage of beta carotene. The F_2 frequency distributions do not support the influence of the beta modifier,

Table 2. χ^2 **goodness-of-fit test for a single, dominant gene model conferring orange versus red fruit pigmentation in tomato populations derived from a cross of *L. esculentum* cv. Floradade × *L. cheesmanii*, LA317**

Population[a]	Observed: Orange	Observed: Red	Total	Ratio tested	χ^2	Probability
P_1	0	10	10	0:1		
P_2	5	0	5	1:0		
F_1	20	0	20	1:0		
BC_1	42	54	96	1:1	1.50	$P > .2$
F_2	98	25	123	3:1	1 44	$P > 2$

[a] See Figure 1 for population descriptions.

$Mo_B{}^+$, from either parental stock in the current study as there was no evidence for three distinct peaks denoting high, intermediate, and low classes in the F_2 distribution frequency (Tomes et al. 1954). Preliminary results (Stommel 1991) utilizing *L. esculentum* cv. Floradade indicate heterogeneity within this cultivar for Mo_B. Goodness-of-fit test for a single dominant gene conditioning orange- versus red-pigmented fruit, and hence increased beta carotene levels in orange-fruited phenotypes, indicated sufficiency of the model ($P > .2$, BC_1 and F_2 generations) to explain the observed segregation patterns (Table 2).

Mode of inheritance for beta carotene in *L. cheesmanii* is similar to that reported for the *B* gene from the green-fruited species *L. hirsutum.* The expression of "*B*-like" genes has also been noted in the green-fruited species *L. chilense* (Manuelyan et al. 1975) and *L. chmielewskii* (Chalukova 1988) upon introgression into *L. esculentum* genetic backgrounds. Orange fruit pigmentation was noted by Rick (1956) in a Galapagos accession of *L. pimpinellifolium.* In contrast with the green-fruited species, where carotenoid biosynthesis is presumably blocked early in the biosynthetic pathway, endogenous expression of this "*B*-like" gene does occur in fruit of *L. cheesmanii* and the *L. pimpinellifolium* accession noted. Close linkage has been reported between orange fruit color and the gene conditioning indeterminate plant habit (sp^+) in *L. cheesmanii, L. pimpinellifolium* (Rick 1956), *L. chilense* (Manuelyan et al. 1975), and *L. hirsutum* (Tigchelaar 1987) and strongly suggests the presence of a similar, *B*-like allele that influences beta carotene content in these species. In *L. cheesmanii,* Rick (1956) reported significant departure from a single gene model for orange versus red fruit pigmentation but postulated single gene control based on close linkage between fruit color and plant growth habit.

In maize, Mangelsdorf and Fraps (1931) demonstrated dosage dependence between the dominant *Y1* allele and the accumulation of provitamin A carotenoids in maize endosperm tissue. The number of *Y1* alleles in the triploid endosperm tissue was positively correlated with the level of provitamin A carotenoids. An analogous mechanism was not apparent in tomato as dominance was unambiguous in all generations.

In the absence of selection, mean total colored carotenoid content in the high beta carotene class has not been increased over that of the red, low beta carotene parent in the populations described. The results suggest that beta carotene is likely produced at the expense of lycopene. This finding is consistent with the analysis by Kohler et al. (1947), wherein carotenoid accumulation in high beta carotene accumulating progeny derived from a cross of *L. hirsutum* with *L. esculentum* was described. The current proposed pathway of carotenoid biosynthesis in tomato (Jones and Porter 1986) demonstrates the conversion of lycopene into beta carotene, thus lending support to these observations.

Total levels of colored carotenoids in the orange-fruited *L. cheesmanii* parent were significantly lower in comparison to the red-fruited *L. esculentum* cultivar. The lower levels of carotenoids present in *L. cheesmanii* were also noted in the F_1 generation. However, overall lycopene + beta carotene levels returned to those typical of the *L. esculentum* parent in the BC_1 and to a lesser extent in the F_2 generations. In the BC_1 and F_2 generations, orange fruit classes exhibited substantial increases in lycopene content relative to the orange-fruited parent (P_2) and red fruit classes displayed analogous increases in beta carotene content relative to the red-fruited parent (P_1). These results indicate an influence of the respective parents on total colored carotene content. Hayman's (1958, 1960) generational means analysis revealed nonsignificant additive and dominance components for total colored carotenoid content ($a = -10.91$, $d = -9.02$; R^2 model $= 0.75$), suggesting that additional genetic components, including epistasis or more complex genic interactions, may influence carotenoid content. Tomes et al. (1954) notes the possible influence of minor modifiers on carotenoid content. The presence of intermediate types in the F_2 population of the current study provides some evidence for the influence of modifier genes or genetic background effects on lycopene and beta carotene content, but additional generations will be required to document their importance.

No evidence is available to indicate that physiological constraints limit total beta carotene concentrations below that of lycopene levels in respective orange versus red phenotypes. Highly pigmented segregants were noted among orange- as well as red-fruited types. In the study reported here, variation for beta carotene content observed in the F_2 generation indicates that selection for lines with beta carotene levels two to three times greater than that present in the high beta carotene parent, *L. cheesmanii,* is possible. Total levels of colored carotenoids appear to be under separate genetic control, independent of carotenoid type.

Major modifiers of total carotenoid content and individual carotenoids have been described (reviewed by Khudairi 1972; Stommel 1992). Two of these modifiers, high pigment (*hp*) and dark green (*dg*), are single gene mutants which arose spontaneously in *L. esculentum.* In contrast with the beta modifier (Mo_B), which controls the relative proportion of lycopene to beta carotene, *dg* and *hp* effectively increase total carotenoid content. Undesirable pleiotropic effects have unfortunately restricted the practical use of *dg* and *hp* (Jarret et al. 1984). The potential contribution of minor modifier genes to carotenoid content has received relatively little discussion. Variation for total colored carotenoid content among tomato varieties, breeding lines, and wild accessions is evident (e.g., Gross 1991; Lee and Robinson 1980; Porter and Lincoln 1950), and illustrates the importance of genetic background on total carotenoid content.

In summary, the results provide evidence for monogenic control of beta carotene content *L. cheesmanii.* Total colored carotenoid concentration (lycopene + beta carotene) appeared to be under separate genetic control and influenced by additional gene modifiers or other genic interactions.

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The Margo (*mar*) Seed Coat Character and the *t mar* Interaction in Common Bean

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The inheritance of the seed coat color and color patterns of PI 527753 and PI 527811 was investigated. Dark color expression is usually restricted to the hilum ring and caruncula stripe zones, and the remainder of the seed is off-white Analysis of testcross and F_2 data demonstrated that PI 527753 carries alleles *t mar V* and PI 527811 carries alleles *t mar v*, where *t* controls partly colored patterns and *mar* controls margo pattern and color effects. The *t mar* combination drastically restricts the area of the seed coat with color expression and is epistatic to the usual color-extending effects of the genes for partly colored seed pattern—*Arc*, *Bip*, *exp*, and *diff*. The hypothesis is proposed that *mar* achieves its effect on seed coat color and pattern by retarding the development of seed coat color with respect to the other plant tissues; thus, the mature seed have "immature" seed coat colors, especially in the regions beyond the margo zone.

In 1989, I began a study of Herbert Lamprecht's seed collection of common beans (*Phaseolus vulgaris* L.), which is now available under the USDA Plant Introduction (PI) accession numbers 527711 through 527878 (168 accessions) and is maintained at Pullman, Washington. Numerous accessions in the collection appear to carry the margo seed coat pattern character controlled by the recessive gene *mar* (Lamprecht 1933, 1951), which (ideally) gives the dark pattern color in the margo region of the seed coat (a wide swath about the hilum) and the light pattern color in the remainder of the seed coat (Figure 1: rows 1 and 2, columns 3 and 5; rows 3 and 4, columns 4 and 5). (See also Table 1.) Two of the accessions, PI 527753 (Lamprecht's V0553) and PI 527811 (Lamprecht's V0884), had a seed coat color pattern that has no explanation in the published literature. There have been no other reports of genetic study of the margo character other than the two by Lamprecht (1933, 1951). This article reports the results of an investigation of the margo character in Lamprecht's collection, with emphasis on the inheritance of the unusual seed coat pattern in PI 527753 and PI 527811.

Materials and Methods

Florida breeding line 5-593 has shiny black seeds and bishops violet flowers, which is the result of seed coat genotype *P C J B V* (Prakken 1970). A genetic tester line was constructed by backcrossing the *v* allele into the recurrent parent line 5-593, and the resulting genetic tester line is designated *v* BC_2 5-593. Line *v* BC_2 5-593 has mineral brown seed coat color and white flowers, which are pleiotropic effects of *v* in the genotype *P C J G B v* (Prakken 1970).

All of the parents used in this investigation—5-593, *v* BC_2 5-593, PI 527742, PI 527753, and PI 527811—carry the seed coat color genes *P C J B* (Bassett MJ, unpublished data). To simplify the presentation throughout the paper, only the genotype at *T*, *Mar*, and *V* will be specified, whereas *P C J B* are understood to be present unless otherwise specified.

Lamprecht line V0471 (PI 527742) carries the recessive allele (*mar*) at the margo locus, according to the genetic notes of the Lamprecht seed collection. PI 527742